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All authors declare that they have no conflict of interest.

Authors contributions:

SF, GK-U and AJ planned the study; SF and AJ collected the data; DM carried out the quantifications of analytes in blood; SF carried out the statistical analyses under supervision by AJ; SF wrote the first draft of the manuscript under supervision of AJ. All authors contributed to, and have approved the final manuscript.

Abstract

Purpose: The thiopurines azathioprine and 6-mercaptopurine are frequently used for remission maintenance in patients with inflammatory bowel diseases. However, there are therapy failures, and it is unclear whether clinical and laboratory parameters can be used to predict thiopurine metabolite concentrations (as a surrogate for adequate remission maintenance therapy) and clinical outcome in these patients.

Methods: In this retrospective analysis of clinical routine patient data multivariate statistical models based on Linear Mixed Models regression and Generalized Estimating Equations logistic regression were developed. The adequacy of the models was assessed using Pearson's correlation and a receiver operating characteristic curve.

Results: This study included 273 patients and 1158 thiopurine metabolite measurements as well as routine laboratory and clinical data. In the statistical models, thiopurine metabolite concentrations and the odds of non-remission based on different clinical and laboratory parameters were computed. Correlation (r^2) between predicted and measured thiopurine metabolites were 0.40 ($p<0.001$) for 6-thioguanine nucleotides and 0.53 ($p<0.001$) for 6-methyl-mercaptopurine nucleotides, respectively. The model for remission classified data sets in remission and non-remission with a sensitivity of 63% and a specificity of 73%. The area under the receiver operating characteristic curve of the model was 0.72.

Conclusions: Although the models are not yet accurate enough to be used in clinical routine, model-based prediction of thiopurine metabolite concentrations and of outcome is feasible. Until more accurate models are developed and validated, traditional therapeutic drug monitoring of thiopurine metabolites in patients with inflammatory bowel diseases under thiopurine therapy stays the best tool to individualize therapy.

Introduction

The therapy with thiopurines such as 6-mercaptopurine (MP) and azathioprine (AZA) has become standard in patients with inflammatory bowel diseases (IBD).[1] Weight adapted dosing in mg/kg body weight is unreliable in adjusting sufficient blood concentrations because of a weak dose-effect relationship and high inter-individual variability of thiopurine pharmacokinetics, namely metabolism.[2] Therefore, the quantification of two thiopurine metabolites in blood has been proposed to optimize thiopurine treatment.[3] While high 6-thioguanine nucleotide (TGN) concentrations are responsible for therapeutic effects and myelotoxicity, the 6-methyl-mercaptopurine nucleotides (MMP) cause hepatotoxicity, but no therapeutic effect.[2, 4]

The most important enzyme in the competing pathways of thiopurine metabolism is thiopurine-S-methyltransferase (TPMT). Its inter-individual variation in activity is mainly based on polymorphisms of the TPMT gene.[5] Since the TPMT phenotype has an impact on the thiopurine metabolism, its screening prior to the start of the thiopurine therapy has been suggested.[2, 6-10] In clinical practice, additional TPMT genotyping to detect specific mutations is recommended if the TPMT enzyme activity is below 35 MTG/g Hb/h.[11, 12]

The goal of therapeutic drug monitoring (TDM) is to ensure therapeutic response and reduce adverse drug events (ADE) by keeping concentrations within a predefined range. There is a broad consent that too high or too low TGN concentrations should be avoided, but the exact figure of both threshold values, and even if such rigid threshold values should be generally used at all, is controversially discussed and remains unclear.[1]

While most studies support TDM[1, 5, 13-15], and two meta-analyses[16, 17] have shown associations between TGN concentrations and clinical outcome, some studies found no advantage of TDM-based dosing compared to a weight-based dosing scheme[18, 19], and Waljee et al.[20] claim that algorithms using age and laboratory values outperform TGN metabolite measurements in predicting clinical response by accuracy and costs. Since TDM is relatively time and work intensive, it would be worthwhile to support, or replace, TDM by other approaches. Several surrogates for TGN concentrations have been proposed, but their clinical utility remained limited.[21-24] Therefore, we performed a retrospective data analysis to assess and quantify the predictive value of different clinical and laboratory parameters on the thiopurine metabolite concentrations and the clinical outcome.

Material and methods

Data of all thiopurine metabolite quantifications, which were carried out at the University Hospital of Zurich (USZ) and interpreted by the Department of Clinical Pharmacology and Toxicology between 01.01.2003 and 30.11.2015, formed the retrospective data set for this study. Data were extracted retrospectively using the USZ patient record database KISIM V.4.933 (Cistec AG, Zurich, Switzerland).

Inclusion criteria for patients were a diagnosis of IBD (Crohn's disease, ulcerative colitis, indeterminate colitis) and at least one thiopurine metabolite quantification at the USZ in the mentioned time frame. Exclusion criteria were a documented refusal of the patient to use his or her data for research purposes and insufficient clinical data. Patient characteristics and clinical parameters such as age, sex, smoking status, BMI, diagnosis, TPMT genotype, TPMT phenotype, IBD concerning co-medication as well as dose, type of thiopurine given (AZA, MP, thioguanine) were documented for each metabolite measurement as far as available. Additionally, the MMP/TGN ratio was calculated. A patient with TPMT activity below 35 MTG/g Hb/h was defined as low enzyme activity phenotype, one with equal or higher activity was defined as intermediate/high enzyme activity phenotype. Also, laboratory parameters prior to or at the moment of metabolite measurement were gathered. The data set contained information about hemoglobin (Hb), mean cellular volume (MCV), platelets, leukocytes, lymphocytes, creatinine, total bilirubin (hereinafter referred to as bilirubin), albumin, transaminases, alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), c-reactive protein (CRP), prothrombin time (PT), and calprotectine. The study was approved by the Ethics Committee of the Canton of Zurich (BASEC-Nr. 2017-02317) and carried out respecting all pertinent laws and regulations in force in Switzerland at the time of data collection. The clinical outcome forms the dependent binary variable of the logistic regression and was therefore categorised in the two characteristics "remission" and "non-remission", whereas "remission" is used synonymously with (therapeutic) response and "non-remission" with non-response, respectively. The classification of the clinical outcome was mainly based on the documented global assessment of the treating physician. If such an information was not available, the following criteria defined the classification. Patients were classified as responders, if they did not suffer from a stool frequency higher than two solid defecations per day or no change in treatment was described. As soon as patients suffered from a poor general condition related to the IBD, blood in stool or more than two fluid defecations, they were classified as non-responders.

For the metabolite measurements, the quantification method used at the Department of Clinical Chemistry of the University Hospital of Zurich was changed during the study period. The high performance liquid chromatography with ultraviolet detection (HPLC-UV),[25, 26] which was used until 06.05.2011 has been replaced by a liquid chromatography–mass spectrometry (LC-MS) quantification method, which has been developed at the USZ based on the work of Dervieux et al.[27] Both fully validated methods have been compared and they have been completely consistent. Statistical analysis was performed using IBM SPSS Statistics 22 (International Business Machines Corp. Armonk, NY, USA). We used frequencies, means, median, standard deviation σ, minimum, maximum and Inter Quartile Range (IQR) for descriptive statistics. The following continuous predictors violated the normal distribution assumption (tested by visual assessment with boxplot diagrams) and were therefore transformed to their natural logarithm ln(y) for all analyses: creatinine, bilirubin, AST, ALT, GGT, ALP, CRP, leukocytes, lymphocytes, calprotectin, TGN, MMP, MMP/TGN. Multiple observations per patient in our data implied clustered data, therefore Linear Mixed Models Regression and Generalized Estimating Equations (GEE) logistic regression were used for all analyses.

To analyse the impact of a single predictor on ln(TGN) and ln(MMP), we used Linear Mixed Models with one predictor. To elicit the statistical model predicting the TGN and MMP concentrations best, Multiple Linear Mixed Models were used. The multiple Linear Mixed Model Regression is based on formula 1.

F.1 $$y_{ij} = e^{a} \times e^{b_1 \times x_{1ij}} \times {x_{2ij}}^{b_2} \times \ldots \times e^{b_K \times x_{Kij}} \times e^{r_{ij}} \times e^{u_i}$$

i: patient number, i = [1, …, 273], j: observation number within patient i, j = [1, …, n_i], k: predictor number k = [1, …, K], y_{ij}; dependent variable, x_{kij}; predictor, a: regression coefficient, intercept, b_k: regression coefficient, slope, r_{ij}: residuum, u_i: random effect, e: Euler's number, mathematical constant, e≈2.718

To analyse the impact of a single parameter on binary clinical outcome we used univariate GEE logistic regression with exchangeable correlation structure. To determine the statistical model predicting the binary clinical outcome we used multiple GEE logistic regression with exchangeable correlation structure. The univariate GEE logistic regression is based on formula 2.

F.2 $$\frac{\pi_i}{1-\pi_i} = e^{a} \times e^{b_1 \times x_{1i}} \times {x_{2i}}^{b_2} \times \ldots \times e^{b_K \times x_{Ki}}$$

i: observation number i, i = (1, …, 1158), π_i: probability of non-response, k: predictor number k = (1, …, K), a: regression coefficient, intercept, b_k: regression coefficient, slope, x_{ki}; predictor, e: Euler's number, mathematical constant, e≈2.718

The parameters of the final model were selected based on the following criteria. First, there had to be a significant ($p<0.05$) effect in the univariate analysis. Second, if a parameter had a significant effect, which was in its characteristics clearly not compatible with current knowledge of physiology, that parameter was not included in the model, assuming that an error of the first kind occurred. Different models for each question were evaluated using both Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) for Linear Mixed Models and the Quasi-likelihood under the independence model Criterion (QIC) for GEE, respectively. Smaller information criterion values indicated statistically better fitting models. Two-sided tests were considered significant when p-value was <0.05. The accuracy and clinical applicability of the statistical models was reviewed by comparing the measured and predicted endpoints of all observations using Pearson's correlation. The performance of the statistical model as binary classifier in response/non-response was tested using a receiver operating characteristic (ROC) curve and calculating the area under receiver operating characteristic curve (AUROC). The determination of the cut-off point was based on the highest Youden's J statistic (maximized sensitivity and specificity). It was determined under the assumption that sensitivity and specificity were equally important.

Results

Patients and Observations. Thiopurine metabolite quantifications were available in 372 patients. Ninety-nine patients were excluded because they either had no IBD diagnosis or sufficient clinical data was not available. A total of 273 patients was hence included in this study. Within this population, 40% of the patients were female. 58% of the patients had Crohn's disease, 42% had ulcerative colitis and one patient (<1%) had an indeterminate colitis. Demographics and laboratory values are shown in table 1 and supplementary table 1. 4% of the patients in which TPMT genotyping was performed (n=136) had a mutation in the TPMT gen. Descriptive statistics of the TPMT phenotyping (activity measurements) is shown in table 1. Genotype and phenotype were discordant in 3 patients (2% of the 135 patients in which both genotype and TPMT activity were investigated). A total of 1158 metabolite measurements (observations) were performed for the 273 patients in this study (mean: 4.2 observations per patient, range 1 - 29). In 53% of the observations, patients were non-smokers. The patients were treated with azathioprine in 80% of the observations, followed by mercaptopurine (18%) and thioguanine (2%). In 0.5% of the observations, the treatment was stopped by the patient prior to the metabolite measurement. In 61% of the observations, patients were treated with at least one comedication against IBD (corticosteroids, mesalazine, allopurinol, monoclonal antibodies inhibiting tumor necrosis factor, calcineurin inhibitors). In 11% of the observations, patients did not take any IBD-related comedication at that time point, and information on comedication was not available in 27% of the observations (supplementary table 1). In 69% of the observations, patients were in remission.

Final Statistical Models. The final adjusted coefficient estimates of the two selected models to predict the ln(TGN) and the ln(MMP) including the information criterion (IC) values are shown in table 2. The adjusted coefficient estimates for the statistical models to predict the natural logarithm of the odds of non-remission including its QIC is shown in table 3.

Formula 3-5 show how dependent variables can be predicted by the identified independent variables (predictors) using multivariate linear (F.3/F.4) and logistic (F.5) Mixed Models. The first model (F.3) is determined to predict the TGN concentration. The second model (F.4) is determined to predict the MMP concentration. Formula F.3 and F.4 are based on Formula F.1. The third model (F.5) is determined to predict the clinical outcome, expressed as the odds of non-remission. Formula F.5 is based on F.2. For reasons of clarity and comprehensibility, the parameter estimates in F.3-5 are rounded to one relevant decimal place. For more accurate results, parameter estimates shown in table 2 and 3 can be used.

F.3 $$TGN = 145 \times e^{-0.02 \times BMI} \times e^{0.0004 \times Dose} \times Bili^{0.2} \times ALT^{-0.1} \times CRP^{-0.02} \times e^{-0.07 \times Hb} \times e^{0.03 \times MCV} \times Leuk^{-0.2} \times Lymph^{-0.06}$$

F.4 $$MMP = 3722 \times e^{0.01 \times Dose} \times Bili^{0.4} \times ALT^{0.5} \times AST^{-0.5} \times ALP^{-0.6} \times CRP^{-0.05} \times e^{-0.2 \times Hb} \times e^{0.03 \times MCV}$$

F.5 $$Odds\ of\ non-remission = 0.5 \times e^{-0.6 \times Alb} \times Leuk^{1.2} \times TGN^{-0.3} \times ALP^{0.5} \times CRP^{0.2}$$

TGN: 6-thioguanine nucleotides, MMP: 6-methyl-mercaptopurine, BMI: Body-Mass-Index, Alb: Albumin, Bili: Bilirubin, AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, CRP: C-reactive protein, Hb: Hemoglobin, MCV: Mean cellular volume, Leuk: Leukocytes, Lymph: Lymphocytes, e: Euler's number, mathematical constant, e≈2.718. All parameters are used in the units given in table 1.

Review of statistical models. Both predicted logarithmically transformed metabolite concentrations were significantly, but weakly correlated with the corresponding measured metabolite concentrations. The correlation coefficient r^2 of TGNs and MMPs was 0.40 and 0.53, respectively. P-value was smaller than 0.001 in both analyses. Scatter plot diagrams are shown in figure 1.

When comparing the outcome model performance with TGN, MMP and the MMP/TGN ratio, the model including TGN turned out to be best. The ROC curve of the best binary model for outcome is shown in figure 2. The best cut-off point to differentiate between remission and non-remission is at the odds of 0.32 with a sensitivity of 63% and a specificity of 73%. The AUROC of the binary statistical model was 0.72.

Discussion

In this study, statistical models were developed which can be used to predict thiopurine metabolite concentrations and the clinical outcome in patients with inflammatory bowel diseases under thiopurine therapy, based on clinical and laboratory parameters. The first two statistical models estimate TGN and MMP concentrations separately, based on BMI, dose, ALT, AST, ALP, bilirubin, CRP, MCV, leukocytes and lymphocytes. The third statistical model estimates the odds of non-remission of a patient based on albumin, TGN concentration, CRP, leukocytes and ALP. Our results show that the predicted metabolite concentrations do correlate with the measured ones, but that there is still unexplained variability. The results also show that a classification in remission and non-remission based on laboratory parameters is possible and should be better than a mere by-chance classification.

Until now, only few other studies have tried to predict thiopurine metabolite concentrations and clinical outcome with statistical models using clinical and routine laboratory data. Waljee et al.[20] developed machine learning algorithms which differentiated clinical responders from non-responders more accurately than TGN measurements alone (AUROC: 0.86 versus 0.59, $p<0.001$). In our study, the statistical model could differentiate non-responders from responders with an AUROC of 0.72, which corroborates the usefulness of a statistical model. In the publication by Waljee and coworkers, there was a great number of independent predictor variables, of which the neutrophil count, alkaline phosphatase, red cell distribution width, age and WBC count were the most important. The inclusion of TGN concentrations as an independent variable led to no significant improvement of the AUROC. While the red cell distribution width and the neutrophils were not gathered in our study, the results concerning ALP and WBC seem to be consistent. In our study, ALP and WBC were amongst the variables with the highest predictive value for the clinical outcome. While the predictive value of WBC for the clinical outcome was described earlier[13], the impact

of ALP has not been investigated in earlier studies. The results of Waljee et al. concerning age and TGN concentrations are inconsistent with our results. In our study, age had no significant predictive value on the clinical outcome, while TGN was amongst the stronger predictors. Gardiner et al. [28] showed that a reduced MMP/TGN ratio is associated with therapy response. In our study, the MMP/TGN ratio did not better predict the clinical outcome than the TGN concentration. Several parameters, especially MCV, WBC and lymphocytes have been proposed in different studies as surrogates for TGN concentration measurements.[21-24, 29, 30] In our study, MCV, WBC and lymphocyte count had a statistically significant predictive value for TGN concentrations, but the effect sizes were too small and the prediction by each parameter alone was too inaccurate for clinical practice.

Some inherent limitations of the present study may have mitigated the predictive power of the models. Since parameters like calprotectin (82% missing), prothrombin time (75% missing) and smoking status (40% missing) were missing in too many datasets, they could not be tested in the model development process. Other parameters which were missing in more than 20% of the observations were TPMT activity (38% missing), albumin (36% missing), bilirubin (28% missing) and AST (46% missing). An analysis of a subgroup with complete data to improve the predictive power of the model was abandoned due to too few suitable datasets. Secondly, the classification of the clinical outcome in remission and non-remission was based on a global assessment by the treating physician, or, if not available, on the criteria mentioned above. An objective disease activity score would have been useful, but the clinical data necessary for such scores was not available in a large part of our population. In 18% of the observations, too few information to assess the clinical outcome was available, which also impaired the validity of the statistical analysis. Thirdly, the co-medication (27% missing) was not part of the statistical model. For the logistic regression GEE, binary predictors were needed. Since the combinations of co-medication were highly variable concerning drugs, doses, times of intake, etc, we could not find an adequate solution to transform the complex co-medications into one or more binary predictors in a reasonable and clinically justifiable way. Fourthly, the assay to quantify the TGN concentrations [26], had been replaced during the observation period by another method.[27] Since complete consistency of the results has been tested and confirmed, the concern of some authors [17] that the missing standardisation of TGN assays is a reason for the heterogeneity of the results of association studies between TGN concentrations and clinical outcome should not be valid for our investigation. Lastly, the models generated in the present study have not been validated in an independent dataset of “test” patients.

In this study, the prediction of metabolite concentrations and clinical outcome was based on a multitude of parameters to summarize clinical and laboratory influences and interdependencies in statistical prediction models. Equations as shown above have not been published before. We could show that each a set of different parameters have statistically significant predictive values for the thiopurine metabolite concentrations and for the clinical outcome. Often, the observed effects of individual parameters are small, and relevant changes in the thiopurine metabolite concentrations or the odds of non-remission occur only if the predictive parameters change considerably. However, an appreciable fraction of variability remains unexplained and

hence unpredicted. Therefore, the accuracy is probably not good enough for clinical applicability. Additionally, the models are not intuitive, especially because of the logarithmic transformation of laboratory and clinical data. That is why this study has no immediate practical implications.

Conclusion. Statistical models were developed which allow predictions about the clinical outcome and the thiopurine metabolite concentrations. However, these predictions are still too inaccurate and too complex for direct incorporation into clinical practice, so that therapeutic drug monitoring in patients with inflammatory bowel diseases under thiopurine therapy remains the best tool to supervise dose management and therapy response. Further research is necessary to optimize the model structures to make them clinically applicable and useful.

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Table 1: Descriptive statistics of quantitative clinical and laboratory parameters per observation used for statistical models[1]

	Valid N (%)	Mean	Median	σ	IQR	Min.	Max.
Age [years]	1158 (100%)	38	35	14.4	18	16	81
BMI [kg/m^2]	951 (82%)	23.5	22.5	4	6	14.3	42.3
Dose [mg]	1056 (91%)	104	100	57.2	100	0	300
TPMT activity [MTG/g Hb/h]	713 (62%)	52	51	14.3	18	24	86
Creatinine [µmol/L]	982 (85%)	76	73	20	19	21	235
Albumin [g/L]	744 (64%)	43	43	3.6	4	27	53
Bilirubin [µmol/L]	832 (72%)	11	9	7.7	7	1	79
AST [U/L]	622 (54%)	25	23	14.8	10	9	169
ALT [U/L]	1024 (88%)	25	19	23.6	14	5	291
GGT [U/L]	951 (82%)	39	18	109.8	16	2	1696
ALP [U/L]	939 (81%)	66	58	46.4	27	10	888
CRP [ng/L]	1024 (88%)	7	2	18.5	4	0.3	278
Hb [g/dL]	1112 (96%)	13.3	13.3	1.5	2	7.3	17.7
MCV [fL]	1112 (96%)	92	91	7	9	59	118
Platelets [10^3/µL]	1112 (96%)	312	300	95.1	114	74	843
Leukozytes [10^3/µL]	1112 (96%)	7	6.6	2.85	3.35	1.8	22.57
Lymphocytes [10^3/µL]	1105 (95%)	1.25	1.15	0.67	0.87	0.13	4.93
Prothrombin time [%]	294 (25%)	102	103	14.2	18	29	127
Calprotectin [µg/g]	206 (18%)	617	289	1086.6	723	19	10114
TGN [pmol/8·10^8 RBC]	1158 (100%)	340	306	193.8	213	48	1435
MMP [pmol/8·10^8 RBC]	1158 (100%)	1807	763	3127	1690	46	46410
MMP/TGN [-]	1158 (100%)	6.69	2.66	11.74	5.62	0.08	93.39

[1] *N: number of observations, σ: standard deviation, IQR: interquartile range, BMI: Body-Mass-Index, TPMT: Thiopurine methyltransferase activity, AST: Aspartate transaminase, ALT: Alanine transaminase, GGT: Gamma-glutamyl transpeptidase, ALP: Alkaline phosphatase, CRP: C-reactive protein, Hb: Hemoglobine, MCV: Mean cellular volume, TGN: 6-thioguanine nucleotides, MMP: 6-methyl-mercaptopurine, RBC: red blood cells, [31]: Methyl-thioguanine per gram hemoglobine per hour*

Table 2: Adjusted coefficient estimates from best fitting linear mixed models for the prediction of ln(TGN) and ln(MMP)[1]

	ln(TGN)			ln(MMP)		
	slope (b)	95% CI	p-value	slope (b)	95% CI	p-value
BMI	-0.018	-0.04;0.001	0.064			
Dose	0.0004	-0.001;0.001	0.43	0.01	0.009;0.014	<0.001
Ln(bilirubin)	0.19	0.1;0.29	<0.001	0.43	0.22;0.64	<0.001
Ln(ALT)	-0.13	-0.21;-0.04	0.005	0.47	0.2;0.75	0.001
Ln(CRP)	-0.02	-0.06;0.02	0.322	-0.05	-0.13;0.04	0.294
Hb	-0.07	-0.11;-0.03	0.001	-0.18	-0.27;-0.1	<0.001
MCV	0.03	0.02;0.04	<0.001	0.03	0.01;0.05	0.002
Ln(leukocytes)	-0.22	-0.36;-0.08	0.003			
Ln(lymphocytes)	-0.06	-0.17;0.04	0.231			
Ln(AST)				-0.47	-0.88;-0.06	0.023
Ln(ALP)				-0.62	-0.98;-0.25	0.001
AIC	971			1125		
BIC	980			1132		

[1]*TGN: 6-thioguanine nucleotides, CI: Confidence interval, Ln(y): Natural logarithm of y, BMI: Body-Mass-Index, ALT: Alanine transaminase, AST: Aspartate transaminase, CRP: C-reactive protein, Hb: Hemoglobine, MCV: Mean cellular volume, GEE: General Estimating Equations, π: probability of non-response, ALP: Alkaline phosphatase, TGN: 6-thioguanine nucleotides, AIC: Akaike Information Criterion, BIC: Bayesan Information Criterion,*

Table 3: Adjusted coefficient estimates from best fitting multiple GEE to predict $\ln\frac{\pi}{1-\pi}$ [1]

	Best fitting GEE		
	slope (b)	95% CI	p-value
Albumin	-0.64	-0.12;-0.009	0.023
Ln(ALP)	0.47	-0.19;1.12	0.16
Ln(CRP)	0.16	0.003;0.32	0.045
Ln(leukocytes)	1.2	0.63;1.79	<0.001
Ln(TGN)	-0.31	-0.66;0.043	0.085
QIC	638		

[1] *GEE: General Estimating Equations, π: probability of non-response, CI: Confidence interval, Ln(y): Natural logarithm of y, ALP: Alkaline phosphatase, CRP: C-reactive protein, TGN: 6-thioguanine nucleotides, QIC: Quasi-likelihood under the independence model Criterion*

Legend to Figure 1:

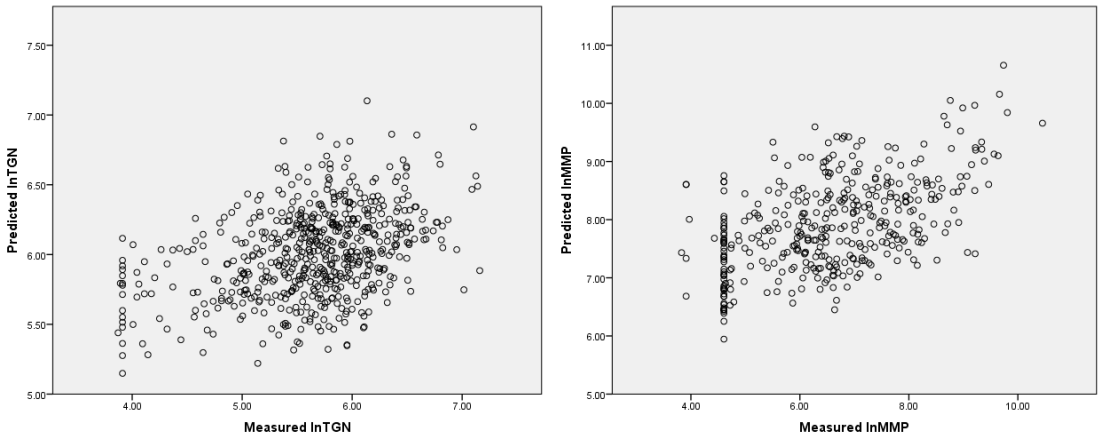
Correlation between measured and predicted thiopurine metabolite concentrations

TGN: 6-thioguanine nucleotides, MMP: 6-methyl-mercaptopurine, ln: Natural logarithm. The vertical lines of dots represent the lower threshold of quantification. Rarely, values below that threshold were documented in the electronic database.

Legend to Figure 2:

Receiver operating characteristic (ROC) curve for the model of treatment outcome

392 **Fig. 1: Correlation between measured and predicted thiopurine metabolite concentrations**



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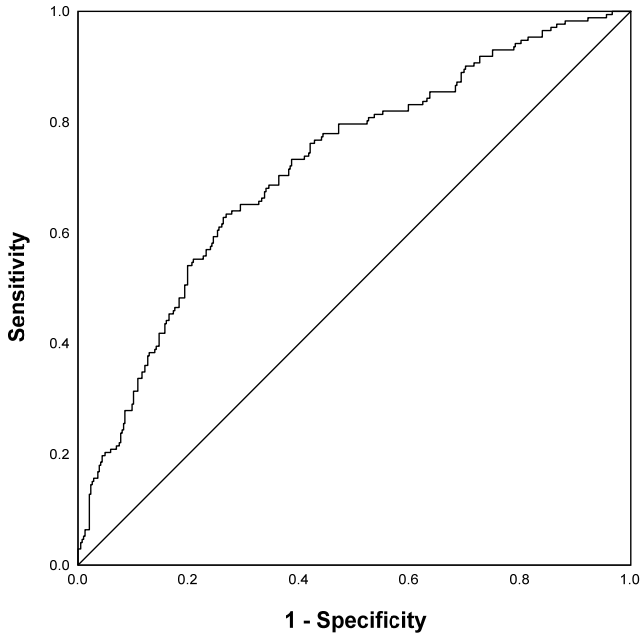
394 **Fig. 2: Receiver operating characteristic (ROC) curve for the model of treatment outcome**



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Do clinical and laboratory parameters predict thiopurine metabolism and clinical outcome in patients with inflammatory bowel diseases?

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Supplementary information:

Supplementary table 1: Descriptive statistics of qualitative data of patients and observations (numbers indicate a positive answer to the question whether the characteristic was present at the time point of observation, numbers in brackets are per cent of patients and observations, respectively. "Valid" means that information on this characteristic was available in the patient records, "missing" means that information was not available).

Patients	273	Observations	1158
Sex (valid 273, 0 missing)		Smoking status (valid 829, 329 missing)	
Female	109 (40%*)	Non-smokers	618 (75%)
Male	164 (60%)	Smokers	211 (25%)
		Type of thiopurine (valid 1119, 39 missing)	
Diagnosis (valid 273, 0 missing)		Azathioprine	893 (80%)
Crohn's disease	157 (58%)	6-mercaptopurine	198 (18%)
Ulcerative colitis	115 (42%)	Thioguanine	22 (2%)
Indeterminate colitis	1 (0%)	None at the time of measurement	6 (0.5%)
		Co-medication** (valid 844, 314 missing)	
TPMT Genotype (valid 136, 137 missing)		No-comedication	133 (11%)
Wild type	130 (96%)	At least one comedication	711 (61%)
Mutation	6 (4%)	Used drugs (valid, missing)	
		Corticosteroids (772, 386)	361 (47%)
TPMT Phenotype (valid 135, 138 missing)		Mesalazine (706, 452)	360 (51%)
Low enzyme activity	9 (7%)	Allopurinol (841, 317)	167 (20%)
High/intermediate enzyme acivity	126 (93%)	Calcineurin inhibitors (1124, 34)	17 (1%)
		TNF inhibitors (1124, 34)	138 (12%)
Mean observations per patient (min; max)	4.2 (1;29)	Clinical outcome (valid 952, 206 missing)	
		Response/Remission	659 (69%)
		Non-response	293 (31%)

*TPMT: Thiopurine methyltransferase, min: minimum, max: maximum, IBD: Inflammatory bowel disease, TNF: Tumor necrosis factor, **only IBD-related co-medication, *complete table presents valid percentage terms*